SHORT COMMUNICATION

An updated linkage and comparative map of porcine chromosome 18

E. M. G. Campbell, S. C. Fahrenkrug, J. L. Vallet, T. P. L. Smith and G. A. Rohrer

USDA, ARS, US Meat Animal Research Center, PO Box 166, Spur 18D, Clay Center, NE, USA

Summary

Swine chromosome 18 (SSC18) has the poorest marker density in the USDA-MARC porcine linkage map. In order to increase the marker density, seven genes from human chromosome 7 (HSA7) expected to map to SSC18 were selected for marker development. The genes selected were: growth hormone releasing hormone receptor (*GHRHR*), GLI-Kruppel family member (*GLI3*), leptin (*LEP*), capping protein muscle Z-line α 2 subunit (*CAPZA2*), β A inhibin (*INHBA*), T-cell receptor β (*TCRB*) and T-cell receptor γ (*TCRG*). Large-insert clones (YACs, BACs and cosmids) that contained these genes, as well as two previously mapped microsatellite markers (SW1808 and SW1984), were identified and screened for microsatellites. New microsatellite markers were developed from these clones and mapped. Selected clones were also physically assigned by fluorescence *in situ* hybridization (FISH). Fifteen new microsatellite markers were added to the SSC18 linkage map resulting in a map of 28 markers. Six genes have been included into the genetic map improving the resolution of the SSC18 and HSA7 comparative map. Assignment of *TCRG* to SSC9 has identified a break in conserved synteny between SSC18 and HSA7.

Keywords mapping, microsatellite, porcine.

The most comprehensive genetic map for SSC18 contained 11 microsatellite markers spanning 57.6 cM (Rohrer *et al.* 1996). SSC18 had the fewest markers and the greatest average interval between markers of any porcine chromosome. Two additional publicly available markers have been added. Bidirectional Zoo-FISH indicates that HSA7p15.2-p12 and HSA7q31-qter are orthologous to SSC18 (Goureau *et al.* 1996). In order to increase marker density large insert clones were identified which contained seven swine orthologs of genes that map to HSA7p15.2-p12 and 7q31-qter. The genes selected were: growth hormone releasing hormone receptor (*GHRHR*), *GLI*-Kruppel family member (*GLI3*), leptin (*LEP*), capping protein muscle Z-line α 2 subunit (*CAPZA2*), β A inhibin (*INHBA*) and T-cell receptor β (*TCRB*) and γ (*TCRG*).

Address for correspondence

Gary A. Rohrer, USDA, ARS, US Meat Animal Research Center, PO Box 166, Spur 18D, Clay Center, NE 68933-0166, USA.
E-mail: rohrer@email.marc.usda.gov

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Initially, porcine YAC and cosmid libraries were screened by polymerase chain reaction (PCR). Reaction products were sequenced to verify that the appropriate gene was amplified. The YAC library was screened as described by Alexander *et al.* (1997) and the cosmid library was screened by an iterative procedure as described by Smith *et al.* (1995). YAC clones were identified for *CAPZA2* and *GLI3* while cosmid clones were initially used for the remaining genes. Subsequently, the RPCI-44 porcine BAC library (BACPAC resources) was screened by hybridization with [α^{32}P] dATP-labelled probes (Fahrenkrug *et al.* 2001) that corresponded to genes for which suitable microsatellites were not detected from the cosmid clones.

Fluorescence *in situ* hybridization (FISH) was used to physically assign one clone obtained for each gene. Clone DNA was fluorescently labelled and hybridized to porcine metaphase chromosome spreads as described by Alexander *et al.* (1996). Two clones were chimeric. The *GLI3* YAC hybridized with both 9p1.1-cent and 18q2.4-qter and the *LEP* cosmid hybridized with both 2pter and 18q2.1. The cosmids for both *TCRB* and *GHRHR* hybridized to pericentric regions of multiple chromosomes. The *TCRG* cosmid

hybridized to SSC9q2.2-q2.3. The *CAPZA2* YAC was assigned to SSC18q2.1-q2.3 and the *INHBA* cosmid hybridized to SSC18q2.4.

Microsatellites were identified from the large insert clones by subcloning and hybridization with a radioactively labelled GT_{11} probe. Microsatellite sequences can be accessed in GenBank (Accession numbers AF391810–AF391826). Primers and conditions for the novel microsatellite markers developed are shown in Table 1. Additionally, microsatellite markers were developed from BACs which contained *SW1808* and *SW1984*. These BACs were identified during characterization of the RPCI-44 library (Fahrenkrug *et al.* 2001). Also included in Table 1 are two microsatellite markers, *SY2* and *SY4*, cloned from a chimeric YAC that contained the *folate binding protein* gene which was assigned to SSC9p2.4 (data not shown). All 15 microsatellite markers were genotyped on 95 animals of the MARC swine mapping population (nine parents, 86 progeny) as described by Rohrer *et al.* (1996). Linkage analysis was performed using CRI-MAP v2.4 (Green *et al.* 1990).

Human chromosome 7 has been reported to be orthologous to SSC9 and SSC18 (Goureau *et al.* 1996) and the current results agree. Figure 1 demonstrates the updated comparative physical maps of SSC18 and HSA7 which indicates that HSA7q31.3-qter represents the proximal half of SSC18 and HSA7p15.2-p12 represents the distal half of SSC18. Table 2 presents the SSC18 linkage group including the 15 new microsatellite markers. This study provides

Table 1 Type of clones, primer sequences, amplification parameters and mapping information of microsatellite markers developed.

Marker	Accession number	Origin	Primers	Annealing temperature	Number of alleles	Heterozygous parents (%)	Location Chr: (cM)
SY2	AF391821	chimeric YAC	tcccccatctttctctctcc agggaggaaataccacagcc	62	8[1]	78	18:3.2
SY4	AF391825	chimeric YAC	tgtaaaagatttaatagcctgcctc tggtttattctttcatgatttcatg	62	6	100	18:3.2
SY25	AF391822	*CAPZA2* YAC	ttgcccctccttccaatc ttccaaactccttatcagtgca	55	3	67	18:35.8
SY31	AF391823	*GLI3* YAC	tagtagctgcacatggtgtaattt ttgtgtaaaaaggtagaaaacgc	55	6	100	18:57.2
SY32	AF391824	*GLI3* YAC	aagaaatattccttgcccagc gcacaatgcagattccacc	55	3	67	18:57.9
SB38	AF391811	*LEP* BAC	cctacccgaggtagacagacc tatagaaccccgaagagagtgc	58	7	89	18:22.8
SB51	AF391812	*GLI3* BAC	agtgcgtgttcggctatg aactctgggtccaatatgaaatc	58	2	44	18:57.9
SB52	AF391813	*GLI3* BAC	cttttgctttcggaaccactta tgtactgcctcattctccacttt	58	2	56	18:57.9
SB53	AF391814	*GLI3* BAC	acccacaccctgagagcactg gctgttccttctgcctgtcca	60	4	67	18:57.8
SB54	AF391815	*GHRHR* BAC	cttggtgtccccgcctgttca tgcgttgctctcattgcctctca	58	2	56	18:44.6
SB55	AF391816	*GHRHR* BAC	tccctctgcccatctcctg cattgaccccatcatgtctgc	55	2	33	18:44.6
SB56	AF391817	SW1808 BAC	gagatgtgaaaatatagcctgtc ccgtcctaaggttgttgaa	58	3	67	18:0.0
SB58	AF391818	SW1984 BAC	acctcacttcccctctgt tttcttattgccacctttcta	55	5	78	18:29.4
SB63	AF391819	*TCRB* BAC	tgatttacaaggttatgttagtg aaacaacaaggtcctactgtat	58	3	44	18:2.1
INHBA	AF391826	*INHBA* cosmid	ctcgtgttctcttaccagaagg acccaggtcgtaaggtatgtc	58	6	78	18:59.5
SW1500	AF391820	*LEP* cosmid	cggggtctaatctacatagctg cacttaaaaagcacctgtaccc	58	7	78	2:2
TCRG	AF391810	*TCRG* cosmid	gaattcaactctcctcaaaggg ctgacaaccctatgtgaaggc	58	7[1]	100	9:96

[1]Number of alleles includes a null allele.

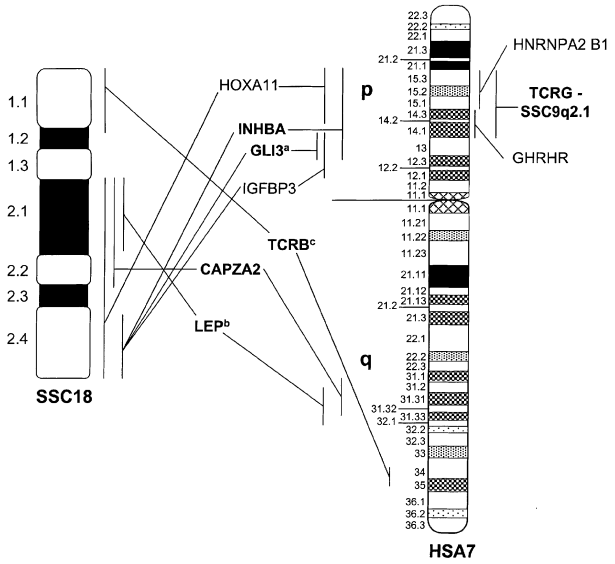

Figure 1 Comparative map of SSC18 and HSA7. (a) The YAC isolated for *GLI3* hybridized with both 9p11.1-cent and 18q2.4-ter. (b) The cosmid for *LEP* hybridized with both 2pter and 18q2.1. (c) The cosmid for *TCRB* hybridized with all acrocentric centromeres, linkage analysis confirmed its location near the centromere of SSC18.

novel porcine assignments for *TCRG* and *GLI3* and a higher resolution comparative map between SSC18 and HSA7. However, these results differ with the published linkage assignment of *LEP* and the physical assignment of *CAPZA2*.

The linkage group reported in Sun *et al.* (1997) had *LEP* (reported as OB) more than 20 cM distal to *S0062*, *GHRHR* and *S0120*. Cepica *et al.* (1999) physically assigned *LEP* to SSC18q1.3-q2.1 with a somatic cell hybrid panel. These two assignments are inconsistent based on the physical assignments reported for *S0062* and *GHRHR*. The *LEP* cosmid identified in this study hybridized to SSC2pter and SSC18q2.1. The FISH signal at SSC18q2.1 is most likely the position of *LEP* as it assigns *LEP* to the same chromosome as previous results. The microsatellite marker isolated from the chimeric *LEP* cosmid, *SW1500*, was assigned by linkage to SSC2:2.1 cM. Because *SW1500* is apparently not associated with *LEP*, a BAC clone was isolated for *LEP* and a microsatellite marker, *SB38*, was developed. This marker placed *LEP* in the SSC18 linkage group at 22.8 cM. The linkage assignment of *SB38* to this position is statistically supported by linkage to *SW1984* (LOD = 22.40, recombination fraction = 0.04) and *SW1023* (LOD = 10.03, recombination fraction = 0.16). While this result is considerably different than the placement of *LEP* in the PiGMaP linkage group, it does concur with the FISH results of this report and the somatic cell hybrid assignment (Cepica *et al.* 1999).

Fridolfsson *et al.* (1997) assigned *CAPZA2* to SSC18q2.4 with a somatic cell hybrid panel. This assignment does not agree with the current FISH results, which placed *CAPZA2* at 18q2.1-q2.2. In the linkage group, the marker for *CAPZA2*, *SY25*, is located proximal to *SW1682*, which has been assigned by FISH to 18q2.3. The genetic assignment is

Table 2 Sex averaged linkage map of SSC18 and human RH information.

Marker	Swine linkage position (cM)	Combined human RH map (cM)[1]
SW1808, SB56	0.0	
SW2540	1.1	
SB63 (*TCRB*)	2.1	154–157.6
SY2, SY4	3.2	
SW1023	4.6	
SB38 (*LEP*)	22.8	131.7–136.4
SB58	29.4	
SW1984	30.1	
SW787	31.8	
SY25 (*CAPZA2*)	35.8	123.9–125.3
S0062	43.5	
SB54, SB55 (*GHRHR*), SJ061, SW1682	44.6	48.8–49.4
S0120	45.3	
HNRNPD (*HNRNPA2B1*)	49.4	40.1–42.1
S0177	55.5	
SWR414, SWR169, SY31	57.2	
SB51, SB52, SB53, SY32 (*GLI3*)	57.9	62.8–66.5
INHBA	59.5	62.8–66.5

[1] The genetic linkage map is merged with radiation hybrid maps from the G3 and GB4 panels (http://www/ncbi.nlm.nih.gov/genemap/map.cgi?CHR = 7). Values presented are in cM from the marker bins used as standards between the two radiation hybrid maps.

supported by linkage to *SW787* (LOD = 7.94, recombination fraction = 0.03) and *S0062* (LOD = 10.64, recombination fraction = 0.08). The linkage data supports a physical assignment proximal to SSC18q2.3 which concurs with the FISH assignment of 18q2.1-q2.2 for *CAPZA2*.

In this study, the cosmid for *TCRB* hybridized to the centromeres of many acrocentric chromosomes including SSC18. *TCRB* was previously mapped by somatic cell hybrids to SSC18 (Rettenberger *et al.* 1996). A physical assignment for *TCRB* at the centromere of SSC18 would be plausible based on the location of SB63 (a microsatellite marker near *TCRB*) at 2.1 cM in the linkage group. The non-specific FISH results could be caused by centromeric repetitive DNA within the cosmid clone. In humans, *TCRB* maps to 7q35, which would indicate that q-arm terminus of HSA7 is orthologous to the centromeric end of SSC18.

In humans, *GHRHR* and heterogeneous nuclear ribonucleoprotein *A2/B1* (*HNRNPA2/B1*) map to 7p14 and 7p15, respectively (gdbwww.gdb.org; Biamonti *et al.* 1994). FISH results for the *GHRHR* cosmid were inconclusive as the clone hybridized to multiple locations. Linkage analysis placed *GHRHR* 4.8 cM proximal of *HNRNPA2/B1*, which was located at 49.4 cM on SSC18 (Wilson *et al.* 2000). The linkage assignment of *GHRHR* is in agreement with the physical assignment of SSC18q2.4 by Sun *et al.* (1997) with a somatic cell hybrid panel.

In humans, *TCRG* also maps to 7p15-p14 and is assigned to the 58.9–60 cM bin on the HSA7 RH map (http://www/ncbi.nlm.nih.gov/genemap/map.cgi?CHR = 7). From this location, *TCRG* would be expected to map to SSC18. On the human radiation hybrid map, *TCRG* is located between *GHRHR* and *GLI3* (a 15-cM interval), both of which mapped to SSC18. However, *TCRG* mapped to SSC9 by both linkage analysis (SSC9 position 96 cM) and FISH (SSC9q2.2–2.3). This identifies a previously undetected break in conserved synteny between HSA7p15-p14 and SSC18.

GLI3 has been physically assigned to 7p13 in humans, whereas the assignment for *INHBA* is 7p15-p13. The *GLI3* YAC clone hybridized to regions of SSC9 and SSC18, both of which were plausible locations based on the comparative map. Two microsatellite markers, *SY31* and *SY32*, isolated from the YAC mapped to the telomeric end of the linkage group of SSC18. In order verify the assignment of *GLI3* to SSC18, microsatellite markers were developed from BAC clones containing *GLI3*. All three markers, *SB51*, *SB52*, and *SB53*, mapped to the same region of SSC18 as *SY31* and *SY32*, confirming the assignment of *GLI3* to SSC18. The physical assignment of *INHBA* to 18q2.4-qter agreed with the previous physical assignment by Lahbib-Mansais *et al.* (1996) and the genetic position of *INHBA* at the distal end of the SSC18 linkage group.

Only 11 markers were on the USDA MARC SSC18 linkage map published by Rohrer *et al.* (1996). Since then, one additional microsatellite marker (*SJ061*, Mikawa *et al.* 1999) and one gene marker (*HNRNPA2/B1*, Wilson *et al.* 2000) have been included. Microsatellite markers associated with six genes have been added to the MARC porcine SSC18 genetic map. The addition of 15 microsatellite markers more than double the number of genetic markers located on SSC18. Despite an average marker interval of 2.2 cM, an 18.2-cM gap between *SW1023* and *SB38* still exists and additional markers in this region of SSC18 would be useful.

The comparative map between HSA7 and SSC18 has also been further defined. The order of genes located on HSA7q is similar to their order on SSC18q and the terminus of HSA7q is apparently orthologous to SSC18 cen. However, rearrangements are evident between HSA7p and SSC18. Furthermore, a break in conserved synteny with respect to human 7p15-p14 has been detected based on physical and genetic assignments of *TCRG* to SSC9. This region of HSA7 was originally believed to be completely orthologous to SSC18. However, these results suggest that a portion of HSA7p15-p14 must be orthologous to SSC9. Further mapping of genes on HSA7p in the porcine genome is warranted to fully understand the chromosomal rearrangements that have occurred over evolutionary time.

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